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Inventor Information for 10/642974

Inventor Name	City	State/Country
MICHAELIS, MARTIN	FRANKFURT	GERMANY
RITZELER, OLAF	BAD SODEN	GERMANY
JAEHNE, GERHARD	FRANKFURT	GERMANY
RUDOLPHI, KARL	MAINZ	GERMANY
GEISSLINGER, GERD	BAD SODEN	GERMANY
SCHAIBLE, HANS-GEORG	JENA	GERMANY

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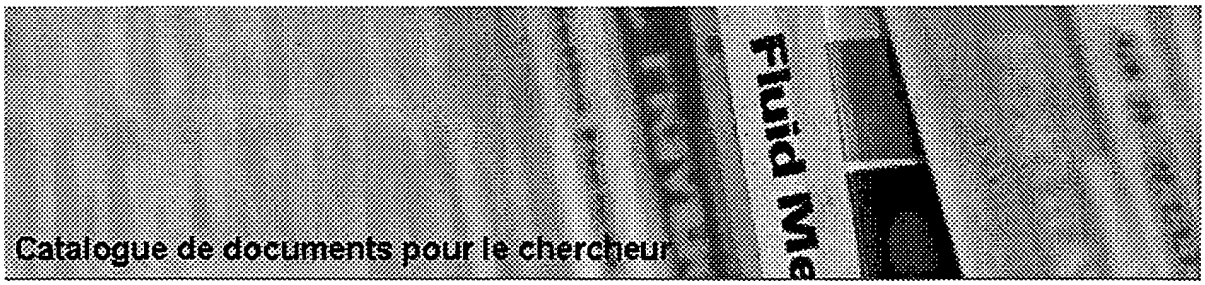

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Spinal NF-kB activation induces COX-2 upregulation and contributes to inflammatory pain hypersensitivity

Auteur(s) / Author(s)
LEE Kyung-Min ; KANG Bong-Seok ; LEE Hye-Lim ; SON Sun-Joo ; HWANG Sung-Hun ; KIM Dong-Sun ; PARK Jae-Sik ; CHO Hee-Jung ;

Résumé / Abstract
Cyclooxygenase-2 (COX-2) is a major contributor to the elevation of spinal prostaglandin E2, which augments the processing of nociceptive stimuli following peripheral inflammation, and dynorphin has been shown to have an important role in acute and chronic pain states. Moreover, the transcription factor, nuclear factor-kappa B (NF-kB), regulates the expressions of both COX-2 and dynorphin. To elucidate the role of spinal NF-kB in the induction of inflammatory pain hypersensitivity, we examined whether activated NF-kB affects pain behavior and the expressions of the mRNAs of COX-2 and prodynorphin following peripheral inflammation. Intrathecal pretreatment with different NF-kB inhibitors, namely, NF-kB decoy or pyrrolidine dithiocarbamate, significantly reduced mechanical allodynia and thermal hyperalgesia following unilateral hindpaw inflammation evoked by complete Freund's adjuvant (CFA). These NF-kB inhibitors also suppressed the activation of spinal NF-kB and the subsequent remarkable elevation of spinal COX-2 mRNA, but not that of prodynorphin mRNA. In addition, the activation of spinal NF-kB following CFA injection was inhibited by intrathecal pretreatments with interleukin-1 receptor antagonist or caspase-1 inhibitor. In view of the fact that interleukin-1 beta (IL-1β) is the major inducer of spinal COX-2 upregulation following CFA injection, our results suggest that IL-1β-induced spinal COX-2 upregulation and pain hypersensitivity following peripheral inflammation are mediated through the activation of the NF-kB-associated pathways.

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REPORT

What Is Nuclear Factor-Kappa Beta?

By Julius G. Goepp, MD

For the past seven years, Life Extension has published extensive articles about chronic inflammation and the numerous diseases it causes, such as cancer, athero-sclerosis, arthritis, dementia, and more.

In these articles, we showed how aging people over-express a molecule called nuclear factor-kappa beta, which then ignites a lethal inflammatory cascade throughout the body.

An abundance of new scientific studies has validated the multiple pathological effects inflicted by nuclear factor-kappa beta. Fortunately, scientists have discovered methods to safely suppress this insidious chronic inflammation-inducing agent. Aging humans are thus able to protect against a major cause of age-related disease.

In this article, we enlighten Life Extension members about what nuclear factor-kappa beta is and what can be done to suppress it.

Understanding the relationship between nuclear factor-kappa beta (**NFkB**) and inflammation is critical to maintaining your health and longevity. Over the last several years, scientists have gained new insights into how **NFkB** functions in the body. As a result, we are on the verge of finding ways to overcome our genetic predisposition toward degenerative conditions'

such as cancer, heart disease, arthritis, and even asthma.

Simply put, **NFkB** is a protein that acts as a switch to turn inflammation on and off in the body. Scientists describe **NFkB** as a "smoke sensor" that detects dangerous threats like free radicals and infectious agents. In response to these threats, **NFkB** "turns on" the genes that produce inflammation. As we age, **NFkB** expression in the body increases, provoking widespread chronic inflammation and setting the stage for diseases ranging from atherosclerosis and diabetes to Alzheimer's. The knowledge of this simple fact should motivate us to counteract **NFkB**'s deleterious effects and thus guard against many of the diseases commonly associated with aging.

As we have reported over the last several years, inflammation is the key initiating factor in major degenerative diseases. In fact, some scientists estimate that inflammation underlies up to 98% of the diseases afflicting humans, including a vast array of seemingly different conditions such as cancer, heart disease, diabetes, and neurodegenerative disorders.1

NFkB is an instigating factor that unleashes inflammatory responses in chronic disease conditions. For example, **NFkB** can signal our cells to continue to multiply long past their normal life span, which can promote cancer. Furthermore, **NFkB** can further spark the smoldering inflammation that damages joint tissues, thereby provoking crippling arthritic conditions. **NFkB** likewise plays a role in spurring inflammation in the nervous system, which can set the stage for the onset of various neurological disorders. Scientists believe that **NFkB**-induced inflammation in the airways may play a role in asthma.

RECENT FINDINGS ON NFkB AND DISEASE

In recent years, numerous studies have shed light on the disease-promoting effects of **NFkB** and the benefits of quieting its activity in the body. For instance, recent studies indicate that **NFkB** plays a role in the following conditions:

- **Autoimmune joint disease:** **NFkB** plays a crucial role in both rheumatoid arthritis and systemic lupus erythematosus, according to Spanish researchers.2 These two autoimmune conditions are known to produce severe joint **pain** and deterioration, as well as other symptoms that dramatically impair quality of life. Effective therapies to block **NFkB** may positively modulate these disease processes.
- **Hepatitis C:** Infection with the hepatitis C virus is a growing cause of liver disease and liver cancer, and (unlike hepatitis B) there is no vaccine to protect against this deadly threat. In early 2006, Japanese scientists determined that **NFkB** plays a key role in the process by which the hepatitis C virus leads to the proliferation of human liver cancer cells.3
- **Inflammatory bowel disease:** Crohn's disease is an inflammatory bowel disease associated with symptoms such as severe abdominal **pain**, diarrhea, weight loss, and rectal bleeding. Recently, scientists noted that therapies that improve the symptoms and pathological signs of Crohn's disease may work by decreasing levels of **NFkB**.4
- **Survival after heart attack:** The death of heart muscle due to a blocked coronary artery is known as a heart attack. If the heart cannot adequately repair itself after such an attack, a common result is heart failure, in which the heart muscle cannot pump enough blood to meet the body's needs. New findings from 2006 suggest that blocking **NFkB** may support cardiac muscle healing and prevent heart failure following heart attack.5
- **Prostate cancer:** Zinc has long been known for its role in supporting healthy prostate function. Research from 2006 suggests that **NFkB** may provide the link between zinc and protection against prostate cancer. Zinc supplementation suppresses **NFkB**'s signaling effects, and researchers believe this may help prevent the metastasis of malignant prostate cancer cells.6
- **Diabetes:** Insulin resistance in muscle tissues is a key factor in type II diabetes. In a recent investigation, researchers studied the muscle tissue of people with type II diabetes and found signs of increased **NFkB** activity. Reducing **NFkB** through exercise training in these individuals led to improvements in blood sugar metabolism.7

The identification of **NFkB** as a critical "switch" that "turns on" inflammation has profound impli-cations for both preventing and treating some of today's deadliest diseases. Clearly, **NFkB** is something we need to control if our goal is to lead a long and healthy life.

Fortunately, ongoing research continues to uncover a wealth of natural remedies that suppress **NFkB**'s activity in the body. These remedies provide the foundation for safe, effective nutritional strategies to quell **NFkB** and disease-provoking inflammation, thus providing a formidable defense against a vast array of deadly diseases and against aging itself.

Interacting with DNA: How NFkB Works

Present in the interior portion (cytoplasm) of every cell, **NFkB** is normally bound to inhibitory proteins that keep it in an inactive state. When cells are exposed to infectious invaders or stressors such as free radicals or environmental toxins (like cigarette smoke), **NFkB** is activated. **NFkB** then travels to the cells' command center, known as the nucleus, where it binds with DNA to turn certain genes on or off. By interacting with more than 400 different genes, **NFkB** can thus activate the body's blueprints for inflammation.[1] These gene products are used to coordinate further inflammatory and immune responses in the body.

NFkB and Cancer Development

One of **NFkB**'s most lethal functions is inducing cancer in our bodies. Scientists are finding that, in addition to its central role in producing inflammation, **NFkB** plays an equally prominent and related role in the development of cancer.

THREE STAGES OF CANCER DEVELOPMENT

Initiation: Cells become cancerous when their DNA is damaged by any of a host of factors, including various forms of radiation, oxidative stress, and specific toxins. Such DNA damage occurs over 3 million times per cell per day. Fortunately, because of cellular repair mechanisms, few of these mutations go on to produce cancer. Cells that survive with enough unrepaired DNA to potentially become cancerous are said to have become initiated.

Promotion: Even initiated cells rarely go on to become cancerous, because cells in most tissues have lost the ability to replicate themselves. The process of programmed cell death, or apoptosis, prevents potentially cancerous cells from passing damaged DNA along to future generations of cells. Unfortunately, under certain circumstances, cells regain the ability to replicate. Such "immortalized" cells are said to have undergone promotion, the second stage in cancer development.

Progression: Even at this late stage, our bodies' defenses normally maintain control even over collections of initiated cells that have undergone promotion. The immune system constantly patrols the body looking for potentially cancerous cells. When it finds them, it destroys these trouble-making cells and mounts an offensive against similar cells found in other body areas. Cancerous tissue that has overcome these defenses is said to be in the final stage of cancer development, known as progression.

Since **NFkB** plays a role in all three stages of cancer development, understanding its actions as well as strategies to control its activity is crucial to both the prevention and adjuvant treatment of various cancers.

NFkB acts in each of the main phases of cancer development, which are known as initiation, promotion, and progression. **NFkB** "switches on" genes that allow cells to become initiated, and once initiated, to have their growth promoted, and once promoted, to progress and invade healthy tissue.[8] Successful cancers evade powerful repair and control mechanisms at each of the three distinct stages of cancer development.[8] Since **NFkB** is involved in each of the three stages, it is critically important that we understand **NFkB**'s actions in our bodies and what we can do to better control them.

The **NFkB** system has emerged as the central actor in the link between inflammation and cancer. **NFkB** affects both malignant and non-malignant tumor cells. In malignant cells, it turns on genes that create resistance to apoptotic cell death and DNA damage, in effect promoting cancer development by rendering cells capable of reproducing, even when they are exposed to pharmaceutical anti-cancer agents. In non-malignant tumor cells, **NFkB** turns on genes that produce factors to stimulate blood vessel formation, in support of rapid tumor enlargement and progression. Finally, byproducts produced by **NFkB** stimulation can also damage DNA, thereby contributing to the very earliest stages of tumor initiation.[8]

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Alleviation of neuropathic pain by intrathecal injection of antisense oligonucleotides to p65 subunit of NF-κB

T. Sun[1,2], W.-G. Song[1,2], Z.-J. Fu[2], Z.-H. Liu[3], Y.-M. Liu[1] and S.-L. Yao[1]*

[1]Department of Anesthesiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China. [2]Department of Pain Management, Shandong Provincial Hospital, Jinan 250021, China. [3]Department of Anesthesiology, The Second Hospital in Shenzhen, Shenzhen 518035, China

**Corresponding author. E-mail: yaoshanglong@eyou.com*

Background. Treatment of neuropathic pain remains a challenge. The current study investigated the therapeutic effect of intrathecal administration of NF-κB antisense oligodeoxynucleotides (ODNs) on mechanical allodynia and thermal hyperalgesia in a chronic constriction injury (CCI) model of rats.

Methods. Lumbar intrathecal catheters were implanted in male Sprague–Dawley rats and a CCI model was established. Thermal and mechanical nociceptive thresholds were assessed with paw withdrawal latency (PWL) to radiant heat and von Frey filaments. The phosphorothioate-modified antisense ODNs to p65 subunit of NF-κB were administered intrathecally on each of five consecutive days post-CCI. Nuclear NF-κB p65 expression was assessed by western blot.

Results. CCI induced mechanical allodynia and thermal hyperalgesia and significantly increased NF-κB p65 protein expression. Intrathecal injection of antisense ODN markedly suppressed the expression of NF-κB p65 protein and significantly attenuated CCI-induced mechanical allodynia and thermal hyperalgesia.

Conclusion. The activation of NF-κB pathway may contribute to neuropathic pain in CCI rats. Suppression of NF-κB could be a potential new strategy for the treatment of neuropathic pain.

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Keywords: antisense oligodeoxynucleotides; complications, chronic constriction injury; NF-κB; spinal cord

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Neuropathic pain, characterized by hyperalgesia, allodynia and spontaneous pain, often occurs as a result of injuries to the peripheral nerve, dorsal root ganglion (DRG), spinal cord or brain. Treatment of neuropathic pain remains difficult. Recently, the NF-κB pathway has been suggested to be involved in chronic neuropathic pain. It was reported that the percentages of activated NF-κB immunoreactive neurons after the partial sciatic nerve injuries in rats in the ipsilateral side of spinal cord to the injury were significantly increased.[1] NF-κB pathway inhibitors, such as pyrrolidine dithiocarbamate, S1627 (IKK inhibitor) and NF-κB decoy, have been used to attenuate chronic pain.[2–4] The antisense strategy is a novel gene therapy technique and could be a useful tool for the study of endogenous gene regulation *in vivo* and *in vitro*.[5] However, few studies have focused on the role of NF-κB antisense ODN in neuropathic pain.

In the current study, we administrated NF-κB antisense ODN intrathecally in a rat chronic constriction injury (CCI) model to investigate whether the regulation of transcription of NF-κB gene is involved in neuropathic pain and to address the feasibility of therapeutic approaches based on specific suppression of the NF-κB pathway.

Materials and methods

Animals

A total of 32 male Sprague–Dawley rats (200–250 g, from the Experimental Animal Center of Shandong University) were kept under a 12 h/12 h light–dark cycle regimen, with free access to food and water. Following the IASP guidelines for pain research in animals, all animal studies were

approved by the Animal Care and Use Committee at the Shandong University and were in accordance with the University's guidelines for the care and use of laboratory animals.

Experimental groups

Rats were randomly divided into four groups (eight rats in each group): the sham group (Intrathecal normal saline, IT NS), CCI group (CCI+IT NS), missense ODN group (CCI+IT missense ODN) and antisense ODN group (CCI+IT antisense ODN).

Intrathecal catheter implantation

Intrathecal catheters were implanted as described by Yaksh and Rudy.[6] In brief, under anaesthesia with chloral hydrate (300 mg kg^{-1}, i.p.), the occipital muscles were bluntly separated, and then the cisternal membrane was exposed. Polyethylene catheters (PE-10) were inserted via an incision in the cisterna magna and advanced 7.0–7.5 cm caudally to the level of the lumbar enlargement. Correct intrathecal placement was confirmed by the dragging or paralysis of bilateral hind limbs after injection of lidocaine 2%, 10 μl. The incision site was closed in layers and the catheter was fixed firmly under the skin and sealed effectively. The rats were allowed to recover for 3 days before the CCI test and monitored daily after surgery for signs of motor deficiency or infection.

Chronic constriction injury

CCI to the sciatic nerve was performed as previously described by Bennett and Xie.[7] Briefly, the rats were anesthetized with chloral hydrate (300 mg kg^{-1} i.p.) and the left sciatic nerve was exposed at the mid-thigh level. Four chromic gut (4–0) ligatures were tied loosely proximal to the trifurcation of the sciatica's at 1.0 mm intervals. Sham surgery was performed by exposing the left sciatic nerve without ligation. The incision site was closed in layers and penicillin was administered i.m.

Drug administration

Intrathecal drug administration was accomplished using a microinjection syringe connected to the intrathecal catheter in awake, gently restrained rats. The injection was performed manually within 30 s using a single injection volume of ODN (20 μg per 10 μl) or normal saline (10 μl) followed by a flush with 10 μl dd-H_2O. Antisense ODN was administered on each of five successive days post-CCI. Normal saline and missense ODN were injected as controls.

Antisense and missense oligonucleotides to the rat p65 subunit were designed according to the cloned 5′ end fragment of the rat p65 gene.[8] Antisense ODN (5′-GGGGA-ACAGTTCGTCCATGGC-3′) and missense ODN (5′-GGGGCGATGAGGCCTACTATC-3′) were synthesized by SBS Genetech Co., Ltd (Beijing, China) and modified with phosphorothioate. Carboxyfluorescein (FAM) was partly labelled to the 5′ end of ODN to examine transfection efficacy by fluorescence microscopy. Synthetic ODN was dissolved in nuclease-free dd-H_2O to a final concentration of 20 μg per 10 μl.

Evaluation of tactile allodynia and thermal hyperalgesia

Mechanical allodynia was assessed with von Frey filaments. The animals, placed on a wire mesh platform and covered with a transparent plastic dome (20 cm × 25 cm × 15 cm), were allowed to acclimate to their surroundings for 30 min before testing. Each filament was applied perpendicularly to the plantar surface of the hindpaw (ipsilateral to the side of surgery in nerve-injured animals). The paw withdrawal threshold (PWT) was determined by sequentially increasing and decreasing the stimulus strength (the 'up-and-down' method), and the data were analysed using the nonparametric method of Dixon, as described by Chaplan and colleagues.[9]

Thermal hyperalgesia was assessed using the paw withdrawal latency (PWL) to radiant heat according to the protocol of Hargreaves and colleagues.[10] Rats were placed in clear plastic cages on an elevated glass plate and allowed to acclimate to their surroundings for 30 min before testing. A high intensity light beam was focused onto the plantar surface of the hindpaw through the glass plate. The nociceptive endpoints in the radiant heat test were the characteristic lifting or licking of the hind paw, while the time to the endpoint was considered the PWL. To avoid tissue damage, a cut-off time of 30 s was used. There were three trials per rat with 5 min intervals between trials. Tests were performed at 1 day before and 1, 3, 5, 7 and 14 days after surgery.

Evaluation of spinal cord ODN uptake[11]

FAM-labelled ODN was injected intrathecally as a single bolus of 20 μg following the CCI procedure. Eight hours after FAM-labelled ODN administration, rats were deeply anesthetized with chloral hydrate and perfused transcardially with 200 ml of phosphate buffered saline (PBS, pH 7.4) containing heparin (1500 iu $litre^{-1}$), followed by 500 ml of cold paraformaldehyde 4%. The L4-5 spinal cord was dissected and cryoprotected with sucrose 30% in nuclease-free PBS overnight at 4°C. Frozen sections (20 μm) were thaw-mounted onto glass slides. Fluorescence images were acquired using an inverted fluorescence microscope. As a control, 20 μg of normal saline was injected intrathecally.

Extracts and western blot analysis

Lumbosacral spinal cord samples were extracted and stored in liquid nitrogen. Nuclear extracts were prepared by adaptation of previously described techniques through freeze/thaw cycles between crushed ice and liquid nitrogen.[12] Briefly, tissue samples were homogenized in ice-cold buffer A (in mmol $litre^{-1}$) [HEPES10; $MgCl_2$

1.5, KCl 10, edetic acid 0.1, egtazic acid 0.1, NaF 50, dithiothreitol (DTT) 1, β-phosphoglycerol 30, Na_3VO_4 1, benzamidine 1, phenylmethylsulfonyl fluoride (PMSF) 0.5, *p*-nitrophenyl phosphate (PNPP) 1 and aprotinin 10, leupeptin 10, pepstatin A 10; pH 7.9]. Proteins were left for 10 min, after addition of 10% NP-40 to a final concentration of 1%, the homogenates were vortexed for 30 s and then centrifuged for 15 min at 800 *g*. The nuclear pellets were resuspended in buffer B (in mmol litre^{-1}) (HEPES 20, NaCl 420, $MgCl_2$ 0.5, edetic acid 1, egtazic acid 1, DTT 1, glycerol 20%, and enzyme inhibitors above, pH 7.9) and then were left for 30 min at 4°C with constant agitation. After centrifugation for 15 min at 12 000 *g*, nuclear extracts were aliquoted and stored at −80°C until use. Protein concentrations were determined using Bradford method. Samples were mixed with loading buffer and boiled for 5 min. Proteins were separated on SDS–PAGE 10% gel, then were electroblotted onto NC membranes (Millipore, Bedford, MA). After blocking for 2 h in PBS with Tween 20 0.1% (PBST) and BSA 3%, membranes were incubated overnight at 4°C with primary rabbit polyclonal anti-p65 (1:1000 Santa Cruz Biotechnology, CA, USA). Membranes were then washed and incubated with secondary antibody (Santa Cruz Biotechnology, California, USA) for 2 h and detected using the NBT/BCIP assay kit.

Statistical analysis

All data are expressed as mean (SD). Statistical analysis was performed using one-way ANOVA or Student's *t*-test. $P<0.05$ was considered statistically significant.

Results

Effects of NB-κB p65 antisense ODN on CCI-induced mechanical allodynia and thermal hyperalgesia

CCI, but not sham surgery, produced significant mechanical allodynia and thermal hyperalgesia ($P<0.05$). The time course of PWT and PWL is presented in Figure 1. Intrathecal injection of NF-κB p65 antisense ODN, not missense ODN, attenuated CCI-induced mechanical allodynia and thermal hyperalgesia ($P<0.05$). The attenuation of allodynia and hyperalgesia was found 1 day after the procedure and persisted through the observation period of 14 days. There was a significant difference in terms of PWT and PWL between the antisense ODN group and the sham-operated group ($P<0.05$).

Uptake of ODN

In order to confirm delivery of ODN into spinal cord cells, antisense labelled with carboxyfluorescein was administrated intrathecally as a single bolus (20 μg) to the lumbar region of the spinal cord. The uptake of labelled ODN by spinal cord cells was evaluated by fluorescence microscopy. No fluorescence was found following intrathecal injection of normal saline (Fig. 2A). Large numbers of cell bodies were highly fluorescent in the group following intrathecal injection of ODN. Fluorescence was observed primarily in the cytoplasm (Fig. 2B). This fluorescence intensity appeared to be variable among the cell bodies irrespective of cell size.

Western blot of NF-κB p65

Effects of the antisense oligonucleotide in inhibiting NF-κB p65 expression were confirmed at the protein level by western blotting (Fig. 3). Compared with the sham group, CCI rats exhibited significantly higher levels of nuclear NF-κB p65 expression ($P<0.01$). The expression of p65 was markedly attenuated after intrathecal injection of antisense ODN ($P<0.05$). No statistical difference was found between CCI group and missense ODN group for nuclear NF-κB p65 expression ($P>0.05$).

Discussion

Results of the current study demonstrated that (i) CCI significantly increased nuclear translocation of NF-κB p65 protein in the spinal cord; (ii) ODN was present in the spinal cord after intrathecal injection of FAM labelled antisense ODN; (iii) expression of NF-κB p65 protein in the spinal cord was significantly down-regulated by administration of antisense ODNs; (iv) intrathecal injection of NF-κB antisense ODN significantly reduced mechanical allodynia and thermal hyperalgesia in CCI animals. Therefore, these results suggest that antisense ODN may alleviate allodynia and hyperalgesia through the NF-κB-related pathway.

NF-κB pathway plays a pivotal role in cell regulation: proliferation, immune cell activation, apoptosis, stress responses, differentiation and oncogenic transformation. Recently, several studies have demonstrated that activation of NF-κB occurs in the DRG and spinal cord, which are both involved in the transmission and processing of nociceptive information. It has been reported that in NF-κB knockout mice the analgesic effects of both low and high frequency electroacupuncture were significantly decreased.[13] Increased NF-κB activation has been observed in rat lumbar DRG neurons after partial sciatic nerve injury[1] and in rat lumbar spinal cord after complete Freund's adjuvant (CFA) was injected subcutaneously into the hindpaws.[14] NF-κB p65 was found to be expressed in a subpopulation (32%) of mixed diameter rat sensory neurons in L4 and L5 dorsal root ganglia, while ipsilateral p65 staining was abolished in a subgroup (60–70%) of these neurons 4 h after crushing the sciatic nerve. The phenomenon was believed because of the failure of retrograde axonal transport of trophic factor(s).[15] Together, these results suggest that spinal cord NF-κB activation is involved, at least in part, in heightened pain states.

The inhibition of NF-κB has been used to attenuate chronic pain states. It has been reported that intrathecal

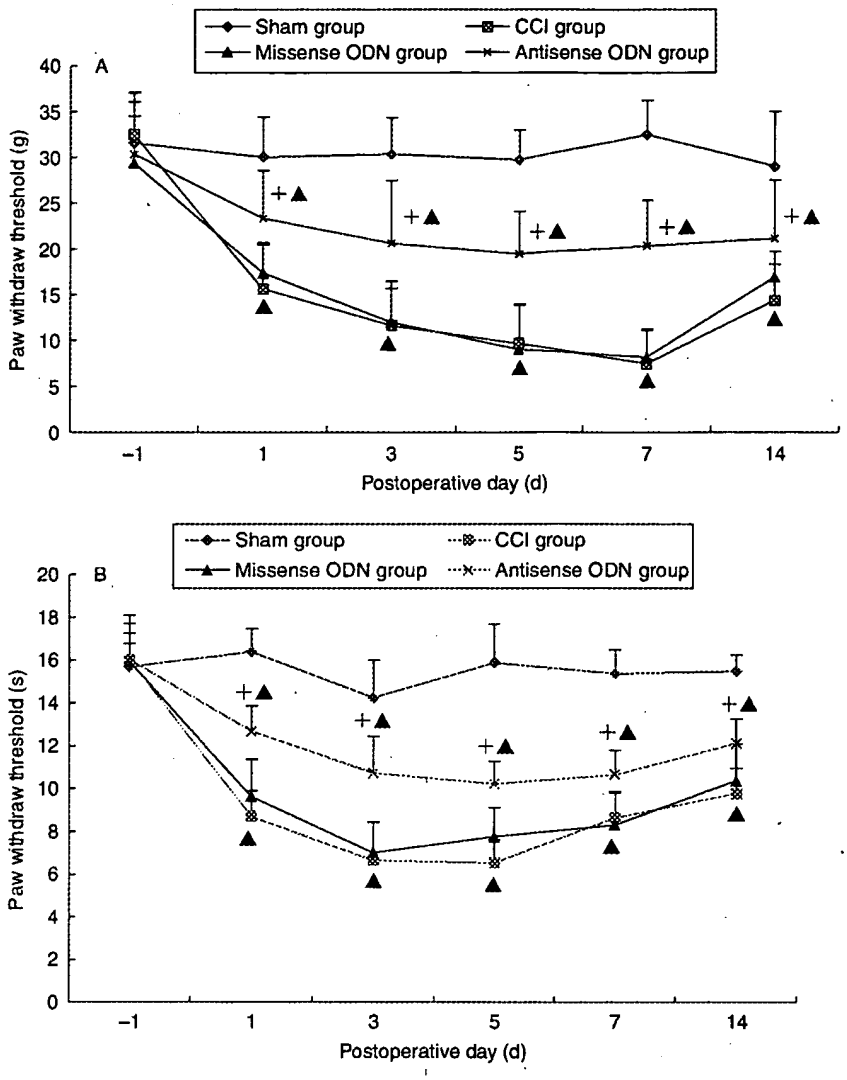

Fig 1 The effects of intrathecal administration of antisense ODNs to p65 subunit of NF-κB on CCI-induced mechanical allodynia (A) and thermal hyperalgesia (B). ($^{\blacktriangle}P<0.05$ *vs* sham group; $^{+}P<0.05$ *vs* CCI group.)

administration of NF-κB inhibitors, pyrrolidinedithiocarbamate (PDTC) and SN50, before gp120 partially alleviated gp120-induced allodynia.[16] Pretreatment with PDTC reduced the development of allodynia induced by intrathecal administration of dynorphin in mice.[17] Also, intrathecal pretreatment with NF-κB decoy or PDTC significantly reduced mechanical allodynia and thermal hyperalgesia after unilateral hindpaw inflammation induced by CFA.[18] These findings are consistent with our results that NF-κB activation is involved, or required, for the induction of allodynia and hyperalgesia.

Several approaches have been described to interfere with the NF-κB pathway. Among them, antisense ODN strategy, which hybridizes with the mRNA strand, is highly specific, as its effect is to inhibit RNA transcription and thereby suppress the synthesis of the gene product. Antisense ODN constitutes a potentially important family of therapeutic compounds for the treatment of a range of diseases.[19] In our study, we used antisense ODN strategy to inhibit the NF-κB pathway. Antisense technologies have been used both *in vitro* and *in vivo* to inhibit expression of NF-κB proteins in a variety of experimental systems. Antisense to the p65 subunit has been shown to greatly inhibit the expression of cell adhesion molecules in endothelial and smooth muscle cells *in vitro*.[20] *In vivo*, the administration of antisense p65 ODN inhibited tumour growth in nude mice, prolongs allo- and xenograft survival and alleviates septic shock in LPS-treated animals.[21,22] It has been reported that intracolonic administration of NF-κB antisense oligonucleotide is effective in ulcerative colitis.[23] As the spinal cord is considered to be one of the key relay stations in the transmission and processing of nociceptive information, intrathecal administration of analgesics becomes an effective mode of pain relief. It has been observed that

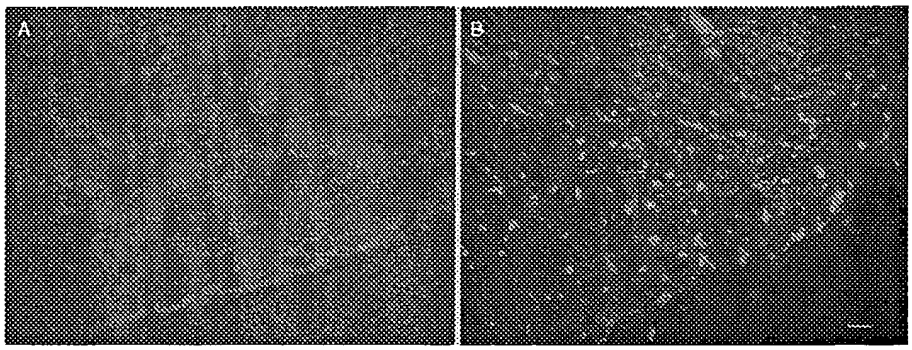

Fig 2 The distribution of FAM-tagged NF-κB antisense ODNs in the lumbar spinal cord after intrathecal injection. Fluorescence micrographs of CCI rat spinal cord from saline control (A) and 8 h after the tagged ODN injection (B). The fluorescence images of the spinal cord were collected using a 40× objective lens and a standardized exposure time of 3 s. Scale bar denotes 50 mm. (A, intrathecal normal saline; B, intrathecal FAM-tagged ODNs.)

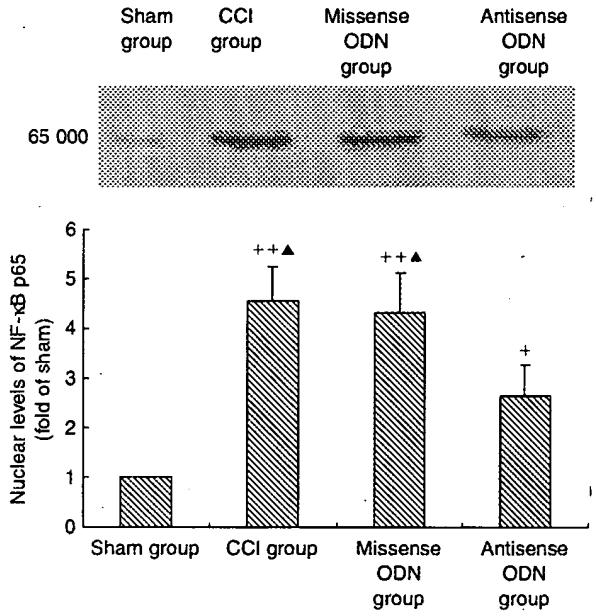

Fig 3 Western blotting showed levels of NF-κB p65 in spinal cord of rats. Rats of sham group exhibited lower levels of NF-κB p65 protein than those of other groups. A significant increase of NF-κB p65 protein was shown in the CCI group. NF-κB p65 protein was down-regulated after intrathecal injection of antisense ODNs, but not missense ODNs. ($^{+}P<0.05$, $^{++}P<0.01$ *vs* sham group; ▲ $P<0.05$ *vs* antisense ODN group.)

intrathecal administration of antisense ODN against targets such as Na(V)1.8, calcium channel alpha2delta-1, mGluR1 and c-fos could alleviate chronic pain states.[24–27] However, little attention has been paid to NF-κB antisense ODN, especially in chronic neuropathic pain models. In the current study, we found that our antisense strategy could effectively block NF-κB activation and alleviate neuropathic pain.

Inflammatory cytokines play important roles in the modulation of neuropathic pain. For example, overproduction of IL-6 is associated with neuropathic pain. Intrathecal injection of human recombinant IL-6 in the absence of nerve injury was able to induce mechanical allodynia in rats and thermal hyperalgesia in the sciatic cryoneurolysis model.[28] Both ipsilateral and mirror-image allodynia in sciatic inflammatory neuropathy (SIN) models of rats were prevented by intrathecal proinflammatory cytokine antagonists specific for interleukin-1, tumour necrosis factor or interleukin-6.[29] Interestingly, these cytokines are proteins down-stream of NF-κB. It has been demonstrated that single endoneurial injection of NF-κB decoy at the site of nerve lesion significantly alleviated thermal hyperalgesia and suppressed the expression of mRNA of the inflammatory cytokines, iNOS, and adhesion molecules at the site of nerve injury.[3] As activated NF-κB mediates the expression of diverse inflammatory and immune response mediators, the inhibition of NF-κB by antisense ODN administration may prevent the onset of thermal hyperalgesia, possibly because of reduced expression of NF-κB regulated inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and IFN-γ; reduced expression of adhesion molecules ICAM-1 and VCAM-1; and chemokines, macrophage inflammatory protein, adhesion factors and proinflammatory enzymes (iNOS, COX-2).[30] However, further study is needed to confirm the linkage between NF-κB and inflammatory cytokines. In addition, the effect of antisense ODN administration on the DRG is still being examined. Further investigations are needed to detail the NF-kB pathway and its relationships between the DRG and the spinal cord.

In summary, the results of the current study suggest that activation of the NF-κB pathway in the spinal cord may contribute to the pathogenesis of neuropathic pain induced by CCI and that suppression of NF-κB protein expression by antisense ODN could alleviate hyperalgesia and allodynia in CCI model in rats. The effect of antisense ODN to p65 subunit of NF-κB is sequence-specific and efficient. It provides not only a potential way for neuropathic pain management but also a method for the study of neuropathic pain.

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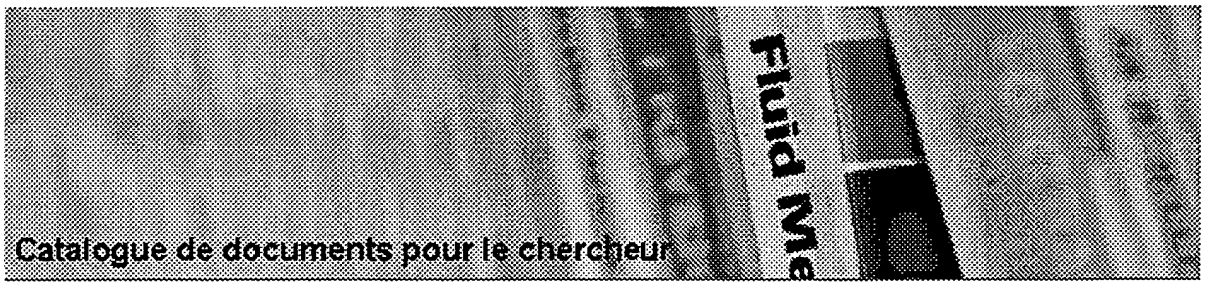

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Transcription factor decoy for NFKB inhibits cytokine and adhesion molecule expressions in synovial cells derived from rheumatoid arthritis

Auteur(s) / Author(s)
TOMITA T. (1) ; TAKANO H. (1) ; TOMITA N. (2) ; MORISHITA R. (3) ; KANEKO M. (1) ; SHI K. (1) ; TAKAHI K. (1) ; NAKASE T. (1) ; KANEDA Y. (3) ; YOSHIKAWA H. (1) ; OCHI T. (1) ;

Affiliation(s) du ou des auteurs / Author(s) Affiliation(s)
(1) Department of Orthopaedic Surgery, Osaka University Medical School, 2-2 Yamada-oka, Suita 565-0871, JAPON
(2) Department of Geriatric Medicine, Osaka University Medical School, 2-2 Yamada-oka, Suita 565-0871, JAPON
(3) Department of Gene Therapy Science, Osaka University Medical School, 2-2 Yamada-oka, Suita 565-0871, JAPON

Résumé / Abstract
Objective. Numerous cytokines are expressed in lesions of synovial hyperplasia of patients with rheumatoid arthritis (RA), and their pathophysiological contributions have been the subject of speculation. These genes are regulated by the transcription factor NFκB which in turn is activated by tumour necrosis factor-α (TNF-α) and cytokines. In this study we examined the inhibition of the production of pro-inflammatory cytokines, adhesion molecule and matrix metalloproteinase (MMP) from synovial tissue of patients with RA by the introduction of synthetic double-stranded DNA with high affinity for the NFKB binding site. Method. NFκB decoy oligonucleotides (ODN) were introduced with the aid of the haemagglutinating virus of Japan (HVJ) liposome method into synovial tissue or synovial cells derived from patients with RA. The levels of interleukin-1 β (IL-1β), IL-6, TNF-α, intercellular adhesion molecule-1 (ICAM-1) and MMP-1 were determined by means of enzyme-linked immunosorbent assay (ELISA) and Northern blotting analysis. A cell counting kit was used to study the effect of NFκB decoy ODN on synovial cell proliferation. Results. The production of these mediators was significantly inhibited by the introduction of NFKB decoy ODN compared with the effect of scrambled decoy ODN. Transfection of NFκB decoy ODN resulted in a significant inhibition of synovial cell proliferation as compared with that of scrambled decoy ODN. Conclusion. The results demonstrated in this study suggest the potential usefulness of NFκB decoy ODN for gene therapy of inflammatory synovitis of RA.

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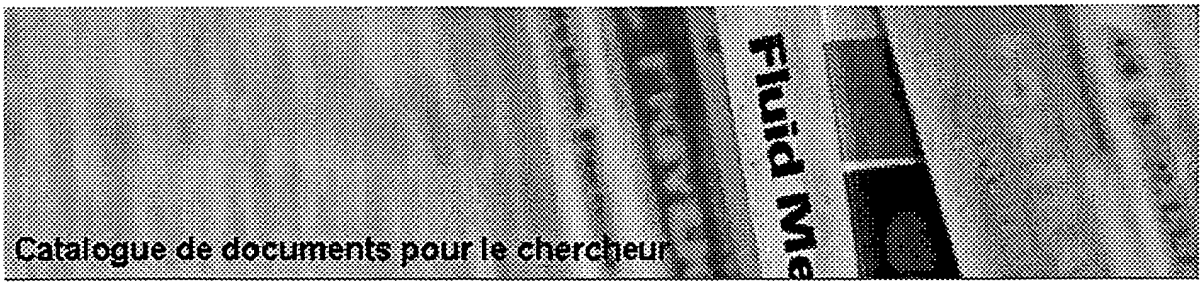

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Elimination of arthritis pain and inflammation for over 2 years with a single 90 min, topical 14% gallium nitrate treatment : Case reports and reviewof actions of gallium III

Auteur(s) / Author(s)
EBY George ;

Résumé / Abstract
Arthritis is inflammation in a joint often with joint damage, usually accompanied by pain, swelling and stiffness, resulting from infection, trauma, degenerative changes, metabolic disturbances, autoimmune or other causes. It occurs in various forms, including rheumatoid arthritis, osteoarthritis, bacterial arthritis and gout. Gallium III can inhibit the production of inflammatory cytokines, such as IL-1beta, produced by macrophage-like cells in vitro. A dose-dependent inhibition of IL-1beta and TPA stimulated MMP activity by gallium nitrate at increasing concentrations occurs, demonstrating that gallium nitrate can be a useful modulator of inflammation in arthritis. Gallium III is an inhibitor of bone resorption and is an effective treatment for hypercalcemia. Gallium III has been reported to be effective in the treatment of mycobacterium butycicum-induced arthritis in rats by antagonism of iron III. Long-term elimination of pain from arthritis by gallium III was first observed in horses primarily being treated for navicular disease. Several people treating their horses with gallium nitrate coincidentally found that arthritis pain in their fingers ended and did not return after soaking their hands in 14% gallium nitrate solution. Therefore, the severely arthritic hands of a 60-year-old woman were topically treated with a 14% aqueous solution of gallium nitrate for 90 min. Pain and inflammation from rheumatoid arthritis diminished rapidly, and neither pain nor inflammation returned during the following 2 years from that single treatment. A 61-year-old woman who had osteoarthritis in her left knee, shoulders and wrists was treated orally with 50 ml of a 1% gallium nitrate solution (120 mg elemental gallium) daily using a two week on and two week off protocol, resulting in almost total elimination of pain while on gallium nitrate, while pain partially returned during the two week off periods. Treatment of frozen shoulder with topical 40% gallium nitrate for 120 min resulted in greatly reduced pain and crepitus almost immediately with complete restoration of range of motion, with pain remaining essentially absent for over 1 year. Mechanisms of action are hypothesized to include anti-inflammatory, bone density improvements, antibacterial, anti-iron III and anti-aluminum III effects. Proper use of gallium III may be effective in terminating pain and inflammation of arthritis for years, often with a single treatment.

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